The effect of exogenous sugar solution and high concentration of CO_2 on the contents of sugar and protein of *Betula platyphylla* leaves

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Abstract: The content of total sugar, sucrose, fructose and protein in the leaves of 3-yr.-old *Betula platyphylla* was measured after the treatment by three exogenous sugar solutions (sucrose, fructose, glucose) and three high concentrations of CO_2 (700, 1 400, 2 100 $\mu L \cdot L^{-1}$) for about a month in 1998. The results showed that spraying three exogenous sugar solutions increased markedly the content of sugar and protein of leaves under 700 $\mu L \cdot L^{-1}$ and 1 400 $\mu L \cdot L^{-1}$ CO_2 The effect of spraying exogenous sucrose solution was the best among the three exogenous sugars. The treatment of spraying exogenous sugar solution and 2 100 $\mu L \cdot L^{-1}$ CO_2 constrained the accumulation of total sugar and protein of leaves. There was no difference in protein content of leaves when spraying glucose and fructose solutions under 700 $\mu L \cdot L^{-1}$ and 1 400 $\mu L \cdot L^{-1}$ CO_2. The treatment of 2 100 $\mu L \cdot L^{-1}$ CO_2 concentration significantly increased the contents of total sugar, sucrose, fructose, and protein of leaves compared with that of the 700 $\mu L \cdot L^{-1}$ and 1 400 $\mu L \cdot L^{-1}$ CO_2 except the plants spraying fructose solution. There was positive correlation between the content of sugar of leaves and CO_2 concentration when spraying same exogenous sugar solution.

Keywords: Exogenous; Sugar; Protein; High CO_2; *Betula platyphylla*
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Introduction

During the early 1990s, the research on white birch mainly focused on the optimal provenance selection (Zhu 2001), breeding, cuttage, etc. in China. In recent years the photosynthetic characteristics (Wang 2001), extraction of genetic material from stamen tissue (Yang 2002), and CO_2 enrichment of the species were also studied. Up to date no report, however, was found on the effect of high CO_2 concentration on the substance content of white birch. Soluble sugar is the main photosynthate in higher plants. Whether the exogenous sugar solution substitutes photosynthate for life substance is still a question. This paper studied the effect of exogenous sugar solution and high CO_2 concentration on the contents of sugar and protein of white birch leaves.

Materials and methods

Experimental materials and design

Three-year-old white birch was planted in three greenhouses at Northeast Forestry University, and 700 $\mu L \cdot L^{-1}$, 1 400 $\mu L \cdot L^{-1}$ and 2 100 $\mu L \cdot L^{-1}$ CO_2 was injected into every greenhouse at the time of 06:00 to 09:00 hour from April 22 to July 15 in 1998. 0.02 M sucrose, fructose and glucose solutions were sprayed on the leaves twice a week from May 5 to July 15. The plant samples in every greenhouse accepted the treatments of three kinds of exogenous sugar solutions. Each kind of soluble sugar treatment had three duplications. Control plants in the greenhouse were only treated by high CO_2 concentration, and control plants outside the greenhouse accepted ambient CO_2 (about 360 $\mu L \cdot L^{-1}$). After treatment of high CO_2 concentration about a month and spraying five-times exogenous sugars solution, the content of sucrose, fructose, total sugar and protein of leaves was measured.

Measurement of soluble sugar and protein

Measuring method referred to the reports by Zhang Zhenqing (1985) and Zhang Zhiliang (1992). Fresh leaves were killed immediately at 105-110°C, then were dried to constant weight at 80°C. Dry leaf of 0.05 g dissolving in 3-mL 80% ethanol was distilled at 80°C for half an hour. After centrifugation, the supernatant was collected. Deposit was duplicated the operation above and all supernatant was rationed to 10 mL.

Measurement of sucrose: the extract of 0.1-mL soluble sugar was mixed with 0.1-mL 30% KOH at boiling water for 10 min, after then it was mixed with 3-mL anthrone solution at 40°C for 10 min, and the optical density of mixture were measured at 620 nm wavelength.

Measurement of fructose: the extract of 0.1-mL soluble sugar was mixed with 3-mL anthrone solution and placed at about 20°C for 90 min, after then the optical density of mixture were measured at 620 nm wavelength.

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Measurement of total sugar: the mixture of extract and anthrone was placed at 90°C water bath for 15 min, after then the optical density of mixture were measured at 620 nm wavelength.

Measurement of protein: Fresh leaf tissue of 0.2 g was ground, and then dissolved in 3 mL phosphoric buffer for three times. After centrifugation, all supernatant was collected and rationed at 10 mL. 0.1 mL distilled solution with 5 mL Coomassic solution was measured at 590 nm wavelength within an hour.

Results and discussion

Effect of 700 $\mu L \cdot L^{-1}$ CO_2 and three exogenous sugar solutions on contents of sugar and protein of leaves

Fig.1 and Fig.2 showed that the contents of sucrose, fructose, and total sugar of control plant in the greenhouse were lower than that accepting the treatments of both exogenous sugar solutions and 700 $\mu L \cdot L^{-1}$ CO_2. The protein content of plant in the greenhouse was significantly lower than that of plant accepting ambient CO_2 out of the greenhouse. For the plants in the greenhouse, the protein content of leaves sprayed with exogenous sugar solution was higher than that only treated by 700 $\mu L \cdot L^{-1}$ CO_2. Though 700 $\mu L \cdot L^{-1}$ CO_2 decreased the protein content of leaves, the decline can be compensated by exogenous sugar solution. At 700 $\mu L \cdot L^{-1}$ CO_2, spraying the exogenous sugar solutions can increase the contents of sugar, protein content of leaves.

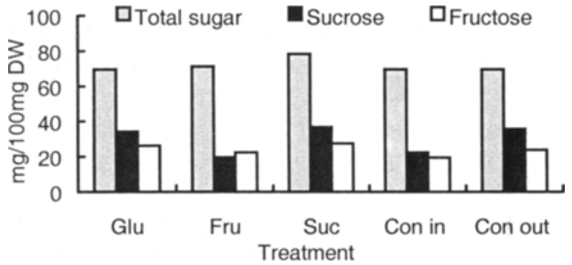

Fig.1 Contents of total sugar, sucrose and fructose of leaves of plant at 700 $\mu L \cdot L^{-1}$ CO_2 concentration.

Glu: Exogenous glucose; Fru: Exogenous fructose; Suc: Exogenous sucrose; Con in: Control in the greenhouse; Con out: Control out of the greenhouse. The following figures are the same.

The content of fructose of the leaves sprayed with exogenous fructose was not the highest. Spraying exogenous sucrose solution significantly increased the contents of total sugar, sucrose, fructose, and protein of leaves. It indicated that specific exogenous sugar solution could not ensure to increase corresponding sugar content of leaves. At 700 $\mu L \cdot L^{-1}$ CO_2 concentration, the effect of spraying exogenous sucrose solution was the best for the accumulation of sugar and protein of leaves. The content of total sugar of leaves sprayed with exogenous sucrose solutions increased by 12% and 10% compared with that of the leaves sprayed with glucose and fructose solutions, respectively. Compared with ambient CO_2, 700 $\mu L \cdot L^{-1}$ CO_2 decreased the contents of sucrose and fructose of leaves but did not decrease the content of total sugar, and 700 $\mu L \cdot L^{-1}$ CO_2 can change the way of carbohydrates reserved in the leaves.

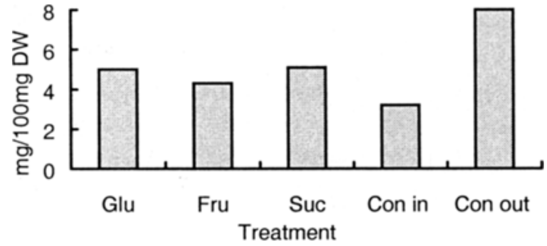

Fig.2 Content of protein of leaves of plant at 700 $\mu L \cdot L^{-1}$ CO_2.

Effect of 1 400 $\mu L \cdot L^{-1}$ CO_2 and three exogenous sugar solutions on contents of sugar and protein of leaves

Contents of total sugar and protein of leaves at 1 400 $\mu L \cdot L^{-1}$ CO_2 concentration showed the same trend as 700 $\mu L \cdot L^{-1}$ CO_2 concentration. Exogenous sugar solution increased the contents of sugar and protein of leaves. 1 400 $\mu L \cdot L^{-1}$ CO_2 still decreased the protein content of leaves. Compared with control plants out of the greenhouse, 1 400 $\mu L \cdot L^{-1}$ CO_2 and three exogenous sugar solutions did not increase the content of sucrose of leaves, but increased the content of fructose. Spraying exogenous sucrose solution increased the content of total sugar of leaves by 42% and 22% compared with that of control plant in the greenhouse and out of the greenhouse, respectively. The contents of sucrose and fructose of plant leaves sprayed with exogenous fructose solution were the highest, but the content of total sugar was the lowest compared with that of the plants sprayed with exogenous glucose and sucrose solutions. The content of total sugar of leaves sprayed with exogenous sucrose solution increased by 4% and 7% compared with that of leaves sprayed with glucose and fructose solutions, respectively. Similarly, protein content increased by 26% and 58%, respectively. To sum up, the effect of spraying exogenous sucrose solutions was still very significant for the accumulations of sugar and protein of leaves at 1 400 $\mu L \cdot L^{-1}$ CO_2 concentration (see Fig. 3 and Fig. 4).

Effect of 2 100 $\mu L \cdot L^{-1}$ CO_2 and three exogenous sugar solutions on contents of sugar and protein of leaves

According to Fig.5 and Fig.6, the contents of total sugar and protein of leaves under 2 100 $\mu L \cdot L^{-1}$ CO_2 concentration was contrary to that under 700 $\mu L \cdot L^{-1}$ and 1 4 0 0 $\mu L \cdot L^{-1}$ CO_2 concentrations. The protein content of plants in the greenhouse was still lower than that out of the greenhouse. Still for the plant in the greenhouse, the protein content of leaves sprayed with exogenous sugar solutions was significantly lower than that of control in the greenhouse. The contents of total sugar of plants sprayed with exogenous sugar solution and plants out of the greenhouse were lower than that of control plants in the greenhouse. It indicated that the combination of 2 100 $\mu L \cdot L^{-1}$ CO_2 and exogenous sugar solutions was not benefit to the accumulations of

sugar and protein of leaves. Spraying exogenous glucose solution has a less constraint for the content of total sugar of leaves, but has a bigger constraint for protein content compared with spraying exogenous sucrose and fructose solutions. The effect of exogenous fructose solution on the contents of total sugar and protein of leaves was totally contrary to the exogenous glucose solution. The content of fructose of leaves sprayed with exogenous sugar solutions was higher than that of control plants. But the content of sucrose of leaves did not present consistent change trend.

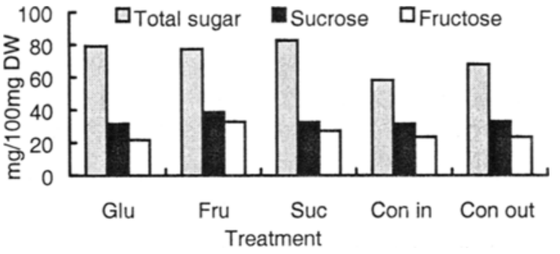

Fig.3 Contents of total sugar, sucrose and fructose of leaves of plant at 1 400 $\mu L \cdot L^{-1}$ CO_2 concentration

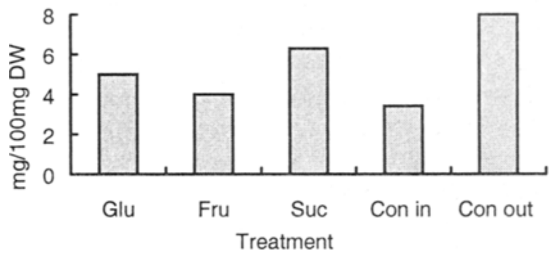

Fig.4 Content of protein of leaves of plant at 1 400 $\mu L \cdot L^{-1}$ CO_2 concentration

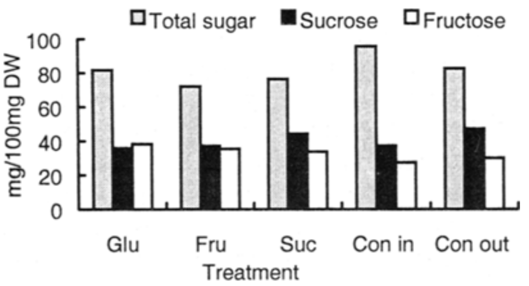

Fig.5 Contents of total sugar, sucrose and fructose of leaves of plant at 2 100 $\mu L \cdot L^{-1}$ CO_2 concentration

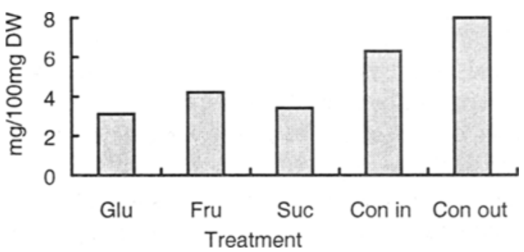

Fig.6 Content of protein of leaves of plant at 2 100 $\mu L \cdot L^{-1}$ CO_2 concentration

There was no significant difference in protein content of leaves between 700 $\mu L \cdot L^{-1}$ and 1 400 $\mu L \cdot L^{-1}$ CO_2 for plants that were not sprayed with exogenous sugar solutions. 2 100 $\mu L \cdot L^{-1}$ CO_2 significantly stimulated the contents of total sugar, sucrose, fructose, and protein of leaves compared with 700 $\mu L \cdot L^{-1}$ and 1 400 $\mu L \cdot L^{-1}$ CO_2 concentrations. There was positive correlation between the content of total sugar of leaves and CO_2 concentration when plants were sprayed with the same exogenous sugar solution.

Organic substance of leaves derives from photosynthesis whose substrate is CO_2. 700 $\mu L \cdot L^{-1}$ and 1 400 $\mu L \cdot L^{-1}$ CO_2 concentrations were not enough to the photosynthesis of three-year-old white birch. White birch still had potential to absorb and utilize exogenous sugar solution. Under 700 $\mu L \cdot L^{-1}$ and 1 400 $\mu L \cdot L^{-1}$ CO_2 concentrations, the effect of spraying exogenous sucrose solution was the best to the substance accumulation of three-year-old white birch leaves compared with spraying exogenous fructose and glucose solution. Sucrose is the main transportation substance and temporary existence form of carbohydrates in higher plants (Xu 2000). The synthesis of sucrose changes with the change of export of photosynthate. Some study reported that exogenous sucrose solution sustained and stimulated stomatal opening of isolated *Victa faba* epidermis (Li *et al.* 2000). Therefore, sucrose solution may be a kind of good external fertilization substance. Under 2 100 $\mu L \cdot L^{-1}$ CO_2, exogenous sugar solution constrained the accumulations of protein and sugar in three-year-old *Betula platyphylla* leaves. Therefore, there is a bound in external supply.

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